Characteristics of Resistance to Columbia Root-knot Nematode Introgressed from several Mexican and North American Wild Potato Species

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Abstract

The Columbia root-knot nematode (CRN), *Meloidogyne chitwoodi* is a serious pest of potato in the Pacific Northwest of the US and in the Netherlands. At present, control is achieved by chemical fumigation, a costly practice. Resistance has been found in several wild species endemic to Mexico and the United States. This type of resistance is expressed as lower root infestation rates and a barrier to the successful establishment of a feeding site, giant cell, and reproduction. In resistant roots, the juveniles remain in a vermiform stage. It appears that localized cell death accompanies the resistance reaction, suggesting the functioning of an R-gene. The inheritance and chromosomal location was identified for two sources of resistance, *Solanum bulbocastanum*, a diploid, and *S. hougasii*, a hexaploid, as the upper arm of chromosome 11. The localization of resistance to the same chromosome suggests synteny and the possible presence of the primitive B genome of *S. bulbocastanum* in *S. hougasii*. Recent surveys have confirmed that two out of 12 plant introduction accessions of *S. fendleri* tested were 100% resistant to race 1 of CRN. Resistance derived from *S. bulbocastanum* was introduced into cultivated potato by protoplast fusion, and a traditional backcrossing program has produced advanced breeding clones with root-knot resistance, good horticultural type, acceptable fry color and long tuber shape.

INTRODUCTION

The Columbia root-knot nematode (CRN), *Meloidogyne chitwoodi* (Golden et al., 1980) is a serious pest of potato in the Pacific Northwest (PNW) of the US and the Netherlands (Evans and Trudgill, 1992; Santo, 1994). The damage threshold is very low, with Santo et al. (1981) having estimated that one nematode per 250 cm^3 soil at planting may lead to crop failure. In the PNW of US, a potato consignment with 10 or more percent tubers blemished due to CRN may be considered by the processors as unmarketable. Fig. 1 shows the damage apparent on an unpeeled tuber of Russet Burbank and the internal blemish that results from juveniles invading the tuber and producing egg masses which appear as brown spots. A tuber is considered a cull if six or more CRN spots are scored, and a strict control of this nematode before planting of potato is essential for crop safety. At present, control is heavily dependent on chemical fumigants that cost up to $700 US per hectare. Clearly enhanced resistance is highly desirable.

There was no resistance known in advanced cultivars, necessitating recourse to wild relatives. Resistance was discovered first in a wild Mexican species *Solanum bulbocastanum* (Brown et al., 1989). As this species is very difficult to hybridize sexually with cultivated potato, somatic hybrids were produced utilizing protoplast fusion (Austin et al., 1993). Subsequent breeding studies reported the recurrent backcrossing and selection of resistance to CRN (Brown et al., 1994, 1995, 1996). Resistance was also found in *S. hougasii* (Brown et al., 1999) and *S. fendleri* (Brown and Mojtahedi, unpublished). The purpose of this report is to describe genetic and breeding aspects of the incorporation of this resistance from these distinct wild species sources.

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MATERIALS AND METHODS

Genetic Materials and Methods

Wild species accessions of *S. bulbocastanum*, *S. hougasii*, and *S. fendleri* were obtained from the USDA/ARS Potato Introduction Station, Sturgeon Bay Wisconsin. Restriction Fragment Length Polymorphisms (RFLP) were analyzed in mapping populations of *S. bulbocastanum* (BC_2) and *S. hougasii* (BC_1) in Southern blots with ^{32}P-radiolabeled probes according to the methods of Bonierbale et al. (1988). In addition, Randomly Amplified Polymorphic DNA (RAPD) markers were established on the linkage map and the frequency assayed in the BC_2 (mapping population) and BC_3 (introgression population) of the *S. bulbocastanum* sources of resistance (Quiros et al., 1993). Linkage groups were first established using the RFLP markers using the tomato map as a guideline (Tanksley et al., 1992). RAPD markers were added to this infrastructure. The comparison of frequency of alien marker between BC_2 and BC_3 was carried out only considering RAPD markers. Mapping algorithms were performed by the program "MAPMAKER" (Lander et al., 1987)

Nematode Inoculation

Resistance to the nematode was determined by inoculation of 5000 eggs of CRN, race 1 (Isolate WAMc1, maintained at the Washington State University, Irrigated Research and Extension Center, Prosser, WA, US). The purity of the inoculum was verified by testing with carrot (cv 'Chantenay Red Core'), a suitable host for race 1 but a non-host for race 2, alfalfa (cv 'Thor'), a suitable host for race 2. Pepper (cv 'California Wonder'), a non-host for CRN, and suitable host for the Northern root-knot nematode, *M. hapla,* was inoculated to test for contamination by this species. Testing on susceptible standards of potato (cv 'Russet Burbank') and tomato (cv 'Rutgers') confirmed the viability of the inoculum. Resistance was expressed as reproductive factor (Rf = final population / initial inoculum), where non-host, poor host and good host status were indicated by $Rf \leq 0.1$, $0.1 < Rf < 1.0$, and $Rf \geq 1.0$, respectively (Oostenbrink, 1966).

Field tests were conducted at the Roza Unit of the Washington State University-Irrigated Agriculture Research and Extension Center in a field where CRN, race 1 has been maintained at high levels for 10 years by alternate year potato and field corn rotations. Clones were planted on May 15 and harvested 4 months later on Sept 15, 2001. Ten tubers each were harvested from six replications of five hill plots, peeled and scored on a scale of 0 to 6, corresponding to nematode sites scored on a peeled tuber of 0, 1 to 3, 4-5, 6-9, 10+, 50+, 100+, respectively (Mojtahedi et al., 1993)

RESULTS AND DISCUSSION

The hybrid derived from somatic fusion between *S. bulbocastanum* and cultivated potato was resistant to the nematode and female fertile, although pollen infertile. It served as the beginning of a backcross program that has proceeded through various generations. The progeny of the BC_2 served as mapping population permitting the positioning of both RFLP and RAPD markers on the 12 linkage groups of potato. The RFLP map established the location of $R_{Mc1(blb)}$ on the upper arm of chromsome 11 distal to the centromere. The BC_3 generation was produced by backcrossing five resistance BC_2 progeny with various cultivate tetraploid recurrent parents. The BC_2 and BC_3 population sizes were N = 63 and 60, respectively. The RAPD's useful in tagging *S. bulbocastanum* were assessed in both BC_2 and BC_3 in order to measure the transmission of alien genome assayed by markers spread over the 12 chromosomes in a quantitative fashion and examine the feasibility of selecting nematode resistant BC_3 progeny with a minimum of extraneous alien genome. The comparison averaged over all the progeny was 44 percent in the BC_2 and 25% in BC_3. The markers on chromosome 8 were present in high proportion in both the BC_2 and BC_3 perhaps indicating that a meiotic aberration occurring in the somatic F_1 hybrid led to a duplex configuration of chromosome 8 from *S. bulbocastanum* in the BC_1 and the consequent high frequency of transmission to the BC_2 seen here. The high transmission to

BC_3 is less easily explained, but might have resulted from some of the five founders being duplex also. The selection of resistant BC_2 as progenitors of the BC_3 also meant a selection for chromosome 11 markers, which are also elevated relative to the rest of the genome in the BC_3 (Fig. 2). A second and different presentation of frequencies is shown in Fig. 3. Here the BC_2 and BC_3 are presented according to the percent of the population falling into different intervals of percent of alien genome averaged over all markers. Interestingly, approximately 7.8 percent of the population has less than 25 percent of the alien genome markers present in BC_2 while 30.3 percent were below this in the BC_3. This information was used to select resistant BC_3 progeny with the least alien genome possible as progenitors of the succeeding backcross population.

A group of BC_4 clones were selected as single-hill, and 12-hill plots in a successive years of field evaluation without nematode resistance assessment. They were planted in a field infested with CRN, race 1 assessed for tuber damage and tested in pots inoculations for Rf value. The results are shown in Fig. 4. Three of the clones depicted to the left, PA98N2-1, PA98N4-2, and PA98N13-3 carry $R_{Mc1(blb)}$, while this factor has not been transmitted to the four clones on the right side of the graph. The nematode reproduction is prolific in the susceptible clones and almost nil in the resistant clones as indicated by the Rf values. Similarly, nematode damage is dichotomous with the resistant clones showing very low values, which would be acceptable as undamaged to processors while the susceptible clones would certainly be classified as culls. These results also indicate that expression of resistance is at a satisfactory level in the BC_4 generation where the genetic background contains the least proportion of alien genome up to this point in the breeding program. The BC_3 progeny PO94A10-3 was genotyped by RAPD markers as a resistant individual containing 14% of the alien genome markers. It is an oblong-shaped tuber with russet skin and a total yield approaching that of the standard cultivar Russet Burbank, although it has poor frying qualities and has a propensity to severe cracking. The resistant clone PA98N13-3 is a derivative of BC_3 progeny PO94A10-3 that was genotyped by RAPD markers as a resistant individual containing 14% of the alien genome markers. It has improved tuber type, high yield and good frying qualities.

Germplasm surveys revealed that *S. hougasii*, a Mexican wild species, also harbored resistance to CRN (Brown et al., 1991). A single hybrid individual was obtained by sexual methods and the subsequent BC_1 generation was used as a mapping population. RFLP's derived from the tomato map Tanksley et al. (1992) were used to localize the resistance. It was found that resistance derived from *S. hougasii* is syntenic to that derived from *S. bulbocastanum*. The marker order is shown in Fig. 5 on chromosome 11 in relation to the nematode resistance loci of both species. Certain markers could not be used in both mapping populations as the panel of restriction enzymes revealed polymorphism in one population but not the other. The synteny suggests that the allohexaploid *S. hougasii* may contain as one of its homeologous genomes, a B-like genome similar to that of *S. bulbocastanum*. The genome of *S. bulbocastanum* is putatively distantly related to the A genome of cultivated potato as evidenced in phyletic studies based on molecular markers and by the strong reproductive isolation exhibited by *S. bulbocastanum* when crossed to most *Solanum* spp. (Debener et al., 1990).

The discovery of resistance to CRN in *S. fendleri* (Janssen et al., 1997) was followed up by our own survey of a core collection consisting of 12 accessions covering the range of distribution of the species. Two of the collections showed non-host level resistance to CRN, race 1 (Fig. 6). The susceptible accessions showed uniformly high Rf values in all the sexual progeny within the seedlot, while every seedling in the resistant seedlots was at the non-host level. All accessions were, however, susceptible to CRN, race 2. The two resistant accessions stem from collections made in two separate mountain ranges in Southeastern Arizona, (i.e., within US territorial borders). Sexual hybrids with resistant *S. fendleri* accessions were made by crossing with cultivated diploid accessions and were screened for resistance. All hybrids were non-hosts, indicating resistance is expressed in a dominant fashion (data not shown). Since these hybrids are triploids, they will be doubled somatically in tissue culture in order to provide a fertile bridging hybrid

that should be intercrossable with cultivated tetraploid potatoes.

CONCLUSIONS

The identification of resistance to CRN in three wild *Solanum* species suggests a common evolutionary origin of these resistances. Synteny between genomic positions in *S. bulbocastanum* and *S. hougasii* adds further support to this. Further studies with the source of resistance from *S. fendleri* may extend this pattern. The inheritance as monogenes and the characteristic nearly complete inhibition of nematode feeding and development in the roots suggest R-genes. Continued examination of these sources of resistance and measuring of the resistance response in different environmental circumstances may reveal distinguishing characteristics in the future that will guide future use in breeding programs to produce resistant potato cultivars. Resistant cultivars will help to reduce the amount of soil fumigation that will be employed in CRN infested fields.

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Figures

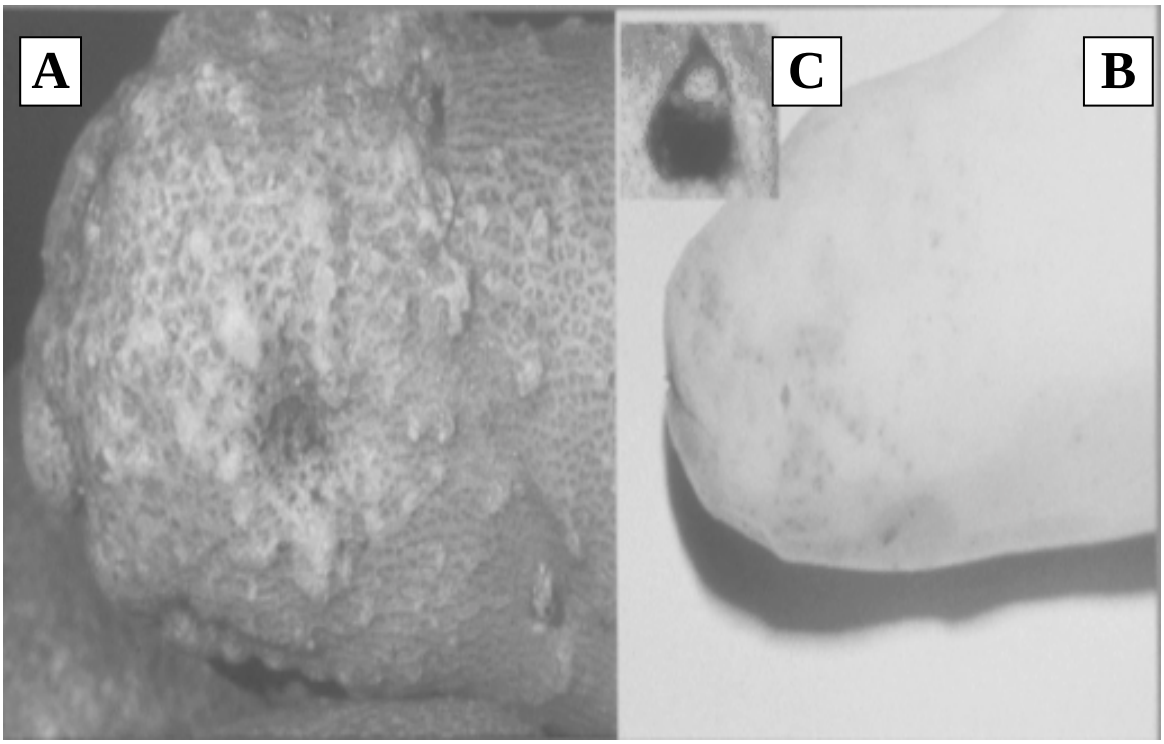

Fig.1. External and internal damage caused by the Columbia root-knot nematode, *Meloidogyne chitwoodi*. In unpeeled cultivar Russet Burbank galling is apparent on tuber surface (A). Infection sites indicate locations of females with egg masses on peeled tuber (B). Tubers with a few infection sites are unmarketable. Female with egg mass dissected from tuber tissue, 60 x (C).

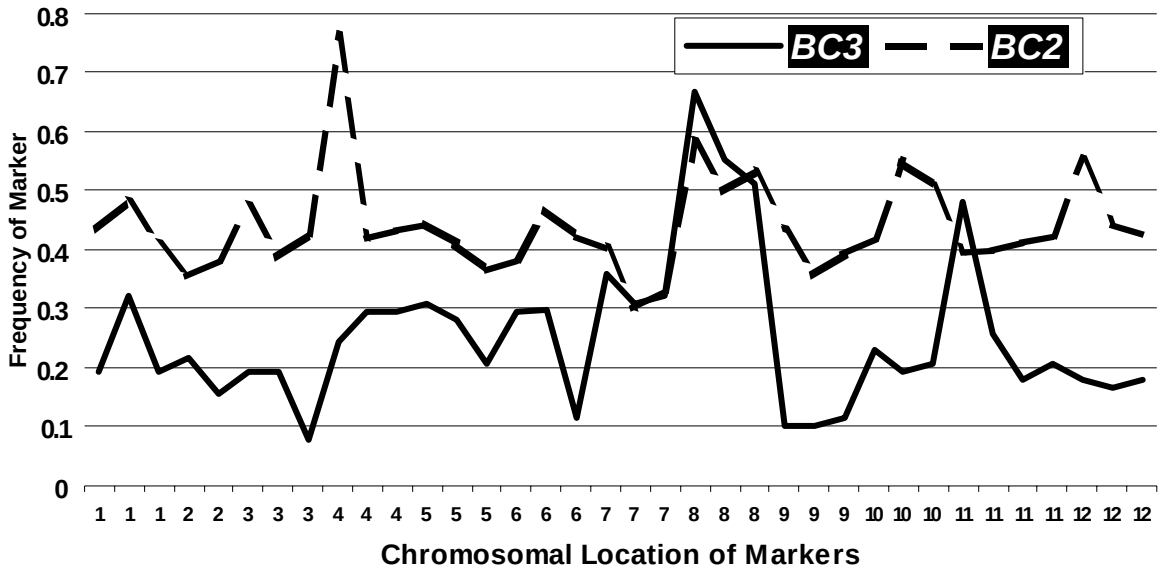

Fig. 2. Distribution of alien genome in BC_2 and BC_3 generations of tetraploid populations introgressing the Columbia root-knot nematode, *Meloidogyne chitwoodi* race 1 resistance from *Solanum bulbocastanum*. Genetic markers are RAPD's localized on chromosomes and specific to the alien genome (i.e., *S. bulbocastanum*).

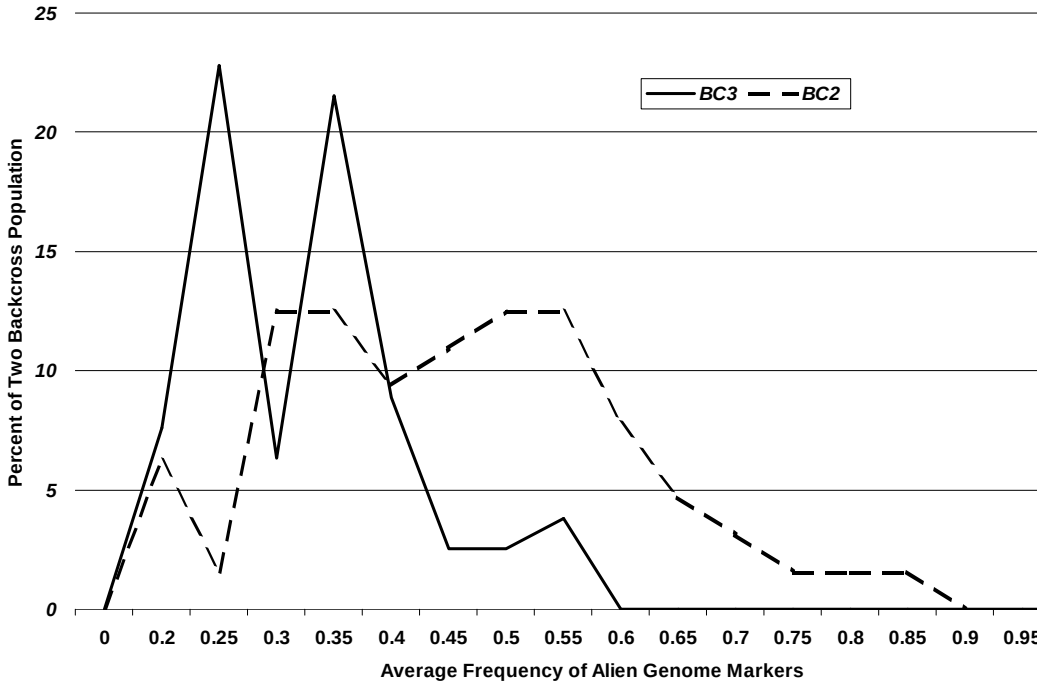

Fig. 3. Distribution of progeny in BC_2 and BC_3 with different content of alien genome marked by chromosome-assigned alien-genome-specific RAPDs.

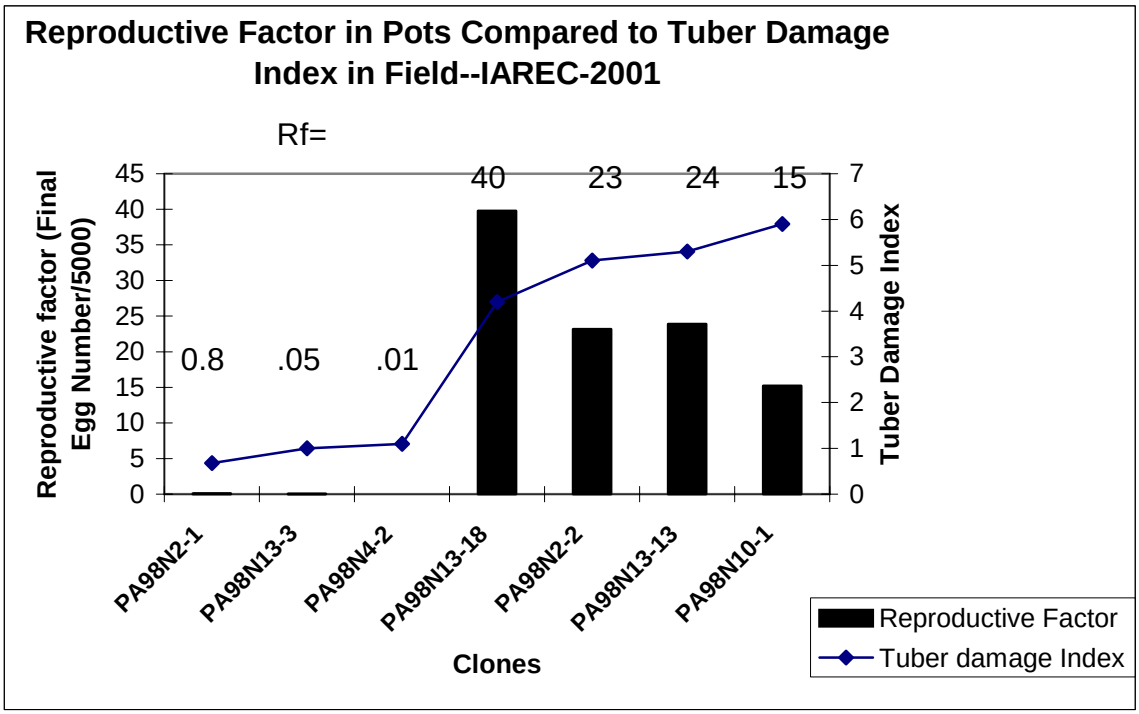

Fig. 4. Relationship of Rf values [Rf = (final egg count / egg count of inoculum)] in pot tests and performance of clones with and without the $R_{Mc1(blb)}$ in the BC_4 of introgression program to introduce resistance to the Columbia root-knot nematode, *Meloidogyne chitwoodi* race 1 derived from *Solanum bulbocastanum.*

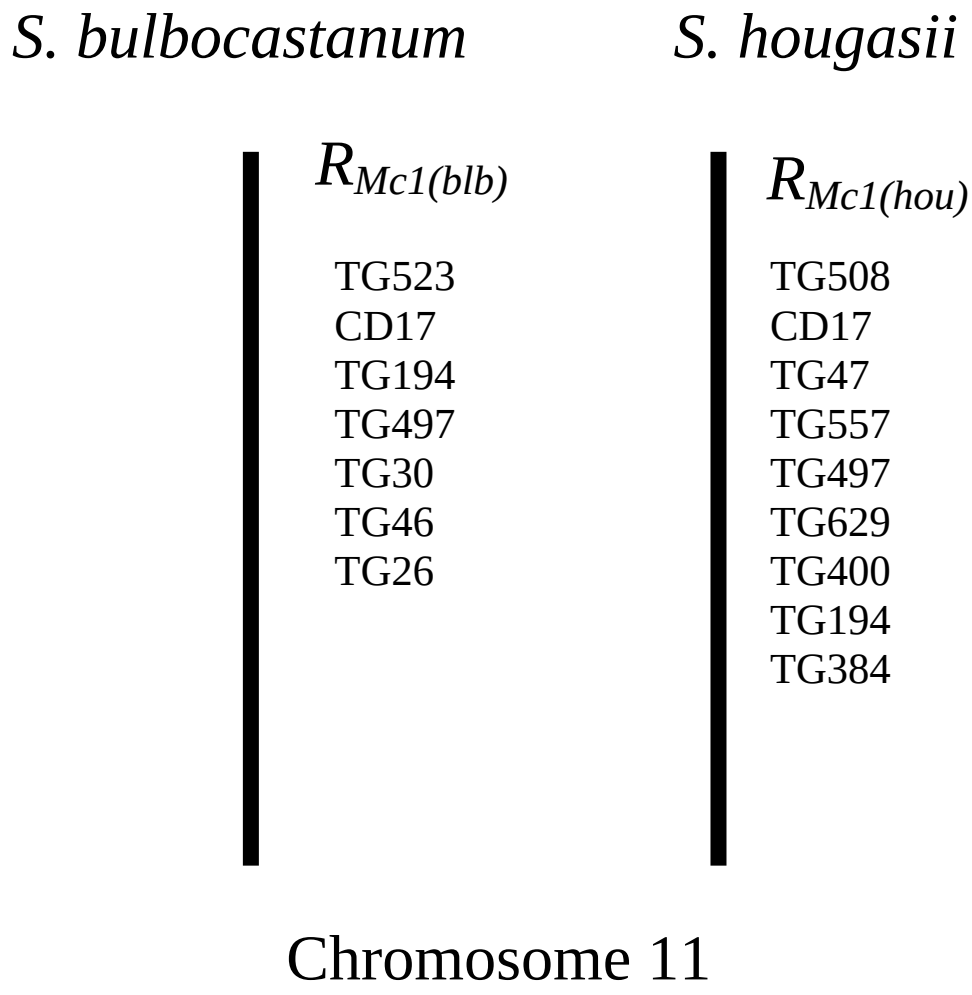

Fig. 5. Mapping order of RFLP markers for the position of resistance genes on chromosome 11 of *Solanum bulbocastanum* and *S. hougasii*.

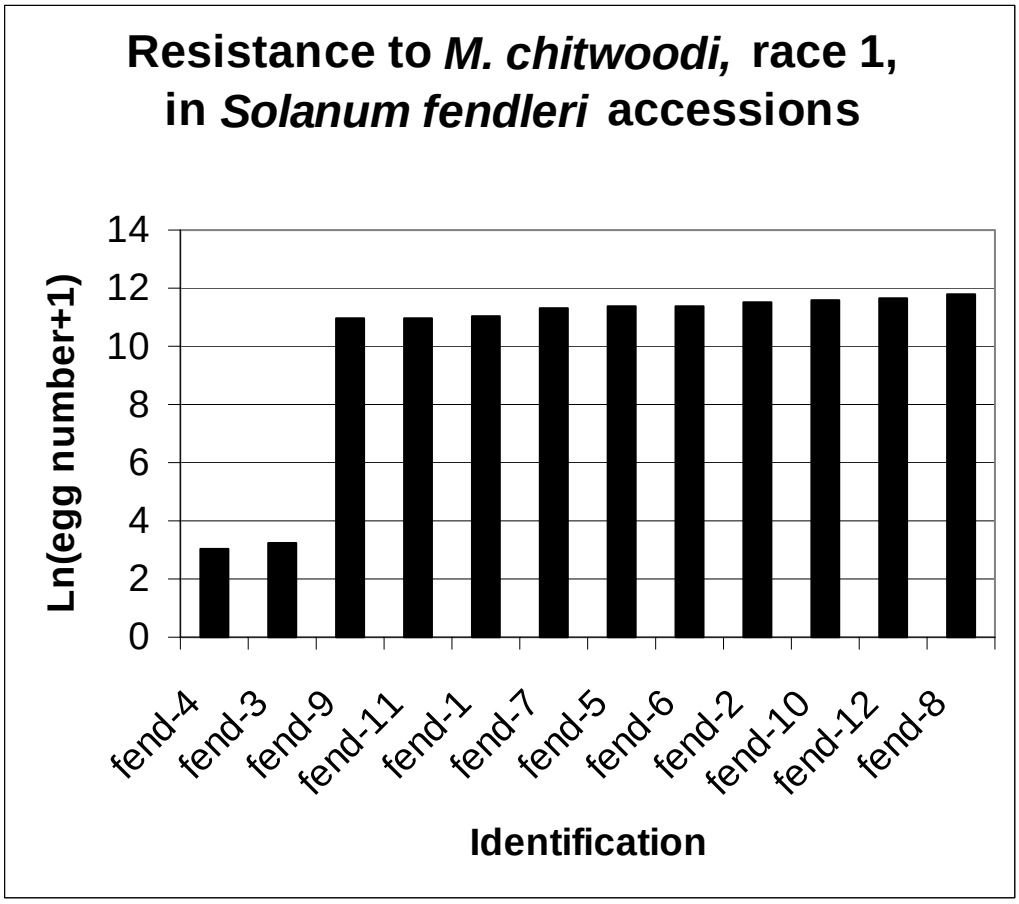

Fig. 6. The natural logarithm of egg counts of pot tests of 12 Plant Introduction accessions of *Solanum fendleri*, representing a core collection. The seedlings of Fen 3 and Fen 4 were uniformly non-hosts (Rf < 0.1) for introduce resistance to the Columbia root-knot nematode, *Meloidogyne chitwoodi* race 1, while all other PI's were uniformly good hosts. (Rf > 1.0).